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60. The vaccine of Claim 12, wherein said recombinantly produced human papillomavirus (PV) L1 protein is not in the form of ~~does not comprise~~ virus-like particles.

61. The method of Claim 19, wherein the recombinantly produced human papillomavirus (PV) L1 protein does not comprise virus-like particles.

62. The vaccine of Claim 50, wherein said recombinant human papillomavirus (PV) L1 protein is not in the form of ~~does not comprise~~ virus-like particles.--

REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.112, and in light of the remarks which follow, are respectfully requested.

By the present amendment, Claim 20 has been cancelled without prejudice and Claim 1 has been amended to obviate the outstanding §112, second paragraph rejection. New Claims 59 through 62 are presented which are directed to additional embodiments of the invention. These claims recite that the recombinant HPV L1 proteins do not comprise virus-like particles. Support for this amendment may be found at page 48, lines 13 to 20, of the subject application. By contrast, the independent claims (from which these claims depend) allow for the recombinant HPV L1 protein to be expressed in any form that provides

for proper conformation. This includes, e.g., virus-like particles and conformationally correct L1 proteins which are not expressed as virus-like particles. By way of example, the disclosed SV40/COS cell expression system apparently does not result in virus-like particles. By contrast, other expression systems (such as the claimed baculovirus expression system) result in virus-like particles which contain conformationally correct L1 proteins. The disclosed results (as well as subsequent experiments considered by the inventors and others) confirm that L1 proteins need not be expressed as particles to confer a protective immune response upon *in vivo* administration. Rather, for efficacy the HPV L1 proteins need only exhibit the same conformational structure as L1 proteins expressed on native, intact HPV viruses. Therefore, both virus-like particles and non-particles which contain conformationally correct HPV L1 proteins are within the scope of the claimed invention.

Turning now to the Office Action, Applicants note that Claims 1-3, 10-26, 46-47 and 50-58 (all of the pending claims) stand rejected. However, prior to specifically addressing the rejections, the recent personal interview with Examiner Caputa is briefly summarized below.

At the outset, Examiner Caputa is thanked for the most helpful personal interview held on October 5, 1995 with the undersigned and Teresa Stanek Rea. During the interview, all of the outstanding rejections were discussed. In particular, the §102 and §103 rejections based on Browne et al *Journal of General Virology* 69(6):1263-1273, Minson U.S. Patent No. 5,045,447,

and Carter et al, *Virology* 182(2):513-521 were discussed at length. It was explained to the Examiner (and indicated that this would likely be further substantiated by a §132 Declaration) that none of these references would teach or suggest the production of conformationally correct L1 proteins. It was further argued that these references would not have inherently produced conformationally correct L1 proteins, notwithstanding the fact that these references disclose expression of L1 proteins in eukaryotic cells, i.e., yeast and mammalian cells.

Specifically, it was explained that both Minson and Browne et al expressed the same defective mutant HPV-16 L1 sequence as was expressed by Zhou et al which does not result in conformationally correct L1 proteins. This argument was supported by reference to the §132 *Journal of Virology*, 68:7260-726 (1994), already of record, as well as Applicants' March 8, 1995 Reply. It was noted that it has already been convincingly established that the mutant HPV-16 L1 sequence disclosed by Zhou et al does not result in the formation of conformationally correct L1 proteins. It was also noted that Zur Hausen was the source of the HPV-16 L1 sequence expressed by Browne et al and Minson (since Minson incorporates Browne et al by reference). It was further asserted that Zur Hausen is a collaborator of Dr. Gissman who was the source of the HPV-16 L1 sequence expressed by Zhou et al. Therefore, Applicants' representatives noted that both Zur Hausen and Dr. Gissman were in possession of and supplied the same mutant HPV-16 sequence. Based on these arguments (which the Examiner suggested should be substantiated by a

declaration), Examiner Caputa graciously indicated that he would likely withdraw the §102 and 103 rejections based on Browne et al and Minson.

The Carter et al reference was also discussed. It was explained why the cloning and expression methods disclosed therein would not give rise to conformationally correct HPV L1 proteins, despite the fact that the authors teach expression of three different HPV-L1 sequences, namely HPV-1, HPV-6 and HPV-16.

With respect to the HPV-16 L1 sequence it was explained that Carter et al also expressed the HPV-16 L1 mutant sequence which was expressed by Zhou et al. Applicants' representatives asserted that this would likely be substantiated by a §132 Declaration.

With respect to the HPV-6b sequence expressed by Carter et al, it was explained that Carter et al expressed a truncated L1 sequence which lacks thirty-nine amino acids of the amino terminal portion of the protein. Moreover, it was noted that this L1 sequence was also fused to another (nonapeptide) sequence. Based on this truncation, and further given the fusion, it was explained that the resultant L1 fusion protein would not be reasonably expected to exhibit proper conformation. It was also noted that subsequent experiments have been conducted by the inventors which confirm that the amino terminal portion of the L1 protein is essential for proper conformation.

Finally, with respect to the HPV-1 L1 sequence expressed by Carter et al it was argued that this also would not have been

expected to give rise to conformationally correct L1 proteins. It was explained that the cloning protocol used by Carter et al was flawed because they cloned their HPV-1 DNA by PCR, but did not take proper precautions to ensure that no mutations were introduced during PCR amplification. With respect to these techniques it was explained that PCR typically gives rise to an error every 300 to 500 nucleotides. Thus, given the size of the nucleotide sequence which was cloned, i.e., about 1500 nucleotides, it was asserted that the cloned HPV-1 sequence would likely contain 3 to 5 point mutations, and possibly more.

Rather, given the fastidiousness of the L1 protein it was explained that one or more of these mutations would reasonably be expected to result in L1 proteins of aberrant conformation. Moreover, it was explained that this can not be established by a side-by-side comparison given the inherent unpredictability of PCR cloning methods.

Essentially, it is impossible to be certain what point mutations were probably introduced during PCR cloning in Carter et al. It is also further impossible to establish *a priori* the effects of any of such point mutations because Carter et al did not sequence their cloned HPV-1 sequence.

Moreover, it was explained that the methodology of Carter et al is further flawed because they did not evaluate the antigenic properties of their recombinant HPV 1 L1 protein using antibodies to determine the conformational integrity of the proteins. Rather, the only antibodies disclosed in the reference are to linear epitopes. It was explained that this is

necessary to determine whether or not a conformationally correct L1 protein was obtained. However, it was asserted that given the fact that PCR generally gives rise to mutations and also because Carter et al took no precautions to determine whether or not such mutations arose and to eliminate such mutations, it is reasonable to conclude (absent evidence to the contrary) that they likely would not have obtained conformationally correct L1 proteins. Moreover, with respect to the method of use and vaccine claims it was argued that Carter et al is further deficient because the reference provides no motivation or incentive to use the disclosed recombinant L1 proteins as an immunogen for providing protection against HPV infection. Rather, the reference only discloses their usage in diagnostic studies. Based on such arguments the Examiner indicated that he would likely withdraw the outstanding §102 and 103 rejections based on Carter et al assuming that these arguments were substantiated by a §132 declaration.

Turning now to the Office Action, it is acknowledged with appreciation that all of the previous art rejections as well as the previous §112 rejections have been withdrawn. However, Claims 20 to 26 stand newly rejected under 35 U.S.C. §112 second paragraph as being indefinite. The Office Action asserts that Claim 20 and the claims which depend thereon lack antecedent basis for the term "animal". This rejection is well taken and Claim 20 has therefore been deleted. Claim 21 has been amended such that it now depends from Claim 19. Thus, withdrawal of the

§112, second paragraph, rejection of Claims 20-26 is respectfully believed to be in order.

Claims 1-3, 10-12 and 15 stand rejected under 35 U.S.C. §102(b) as being anticipated by, or, in the alternative, under 35 U.S.C. §103 as being obvious over Browne et al, (*Journal of General Virology* 69(6):1263-1273)). This rejection is respectfully traversed.

Essentially, the basis of the rejection is that Browne et al would inherently produce conformationally correct HPV-16 L1 proteins. However, as discussed *supra*, Browne et al does not teach or suggest the production of conformationally correct HPV-16 L1 proteins. Nor do they inherently obtain conformationally correct L1 proteins. Instead, this reference describes the expression of a mutant HPV-16 L1 protein using a vaccinia expression system which does not give rise to conformationally correct L1 proteins. More specifically, the authors in Browne et al express the same prototype HPV-16 sequence which had been expressed by Zhou et al *Journal of Virology* 185:251-257 (1991). This is clear given the source of the HPV-16 DNA which they expressed. In particular, Browne et al obtained their HPV-16 L1 DNA from Dr. Zur Hausen (*see* page 1264 of the reference) who is a collaborator of Dr. Gissman who was the source of the defective mutant HPV-16 L1 DNA expressed by Zhou et al (Id.).

As discussed above, convincing evidence has been presented which establishes that this defective mutant sequence does not give rise to conformationally correct L1 proteins upon expression. In support thereof, Applicants respectfully refer the

attention of the Examiner to the March 8, 1995 Reply, specifically at pages 27 through 30, as well as the §132 of A. Bennett Jenson submitted with said Reply and Roden et al, *Journal of Virology* 68:7260-7266 (1994). As may be appreciated upon review thereof, this prototype L1 sequence is derived from an HPV-16 genome which integrated into the chromosome of cervical squamous cell carcinoma which was originally reported by Dürst et al *Proc. Natl. Acad. Sci., USA*, 80:3812-3815 (1983). This prototype HPV-16 differs from the wild-type HPV 16 because it contains a point mutation at position 202 which changes an aspartic acid residue to a histidine residue in the protein product. Moreover, this single modification causes substantial adverse effects on the conformation of the resultant HPV-16 L1 proteins. More specifically, as discussed in the March 8, 1995 Reply, this mutant HPV-16 L1 sequence does not result in conformationally correct L1 proteins suitable for use as a vaccine. This may be appreciated, e.g., from the last page of Roden et al wherein the authors note that antisera raised to particles obtained by expression of the HPV-16 prototype failed to prevent binding of the wild-type HPV-16 virus-like particles to cell surfaces.

Therefore, given the fact that Browne et al expressed the same mutant sequence as did Zhou et al, the rejection based on this reference should be withdrawn for substantially the same reason as the previous §103 and §102 rejections based on Zhou et al.

Turning now to Minson, this patent is cited because of its disclosure relating to the expression of HPV-16 L1 DNA. Based

on this fact, the Examiner concludes that the patentee would inherently obtain conformationally correct L1 proteins. However, this is respectfully traversed. Applicants respectfully advise that the patentee expressed the same mutant HPV-16 DNA sequence expressed by Browne et al as well as Zhou et al. This is clear, e.g., based on the fact that the patentee incorporates by reference the cloning procedures described by Browne et al. In this regard, *see* Col. 5, lines 15 to 17, of the patent. The fact that they expressed a mutant HPV-16 sequence is further clear because the identified source of the HPV-L1 open reading frame is Seedorf et al, *Virology* 145:181 (1985). Applicants respectfully note that the authors in Seedorf et al include Zur Hausen. Therefore, it is quite clear that Minson expressed the same mutant HPV-16 L1 sequence expressed by Zhou et al.

As discussed above, this sequence does not give rise to conformationally correct L1 proteins. This is clear from the §132 Declaration of Dr. Schlegel and Dr. Jenson which is submitted herewith, as well as the previous Declaration by A. Bennett Jenson. Thus, based on the foregoing, withdrawal of the §102 and §103 rejections based on Minson is respectfully requested.

Claims 13, 14, 16-26, 46, 47 and 50-58 further stand rejected under 35 U.S.C. §103 as being unpatentable over Browne et al as applied to Claim 1-3, 10-12 and 15 above and further in view of Danos et al. Browne et al has been discussed *supra*. For the reasons set forth above (and as further established by the §132 Declaration of Drs. Schlegel and Jenson submitted herewith), Browne et al fails to teach or suggest recombinant con-

formationally correct HPV L1 proteins. To the contrary, Browne et al express the same defective assembly mutant HPV-16 L1 sequence expressed by Zhou et al which does not give rise to conformationally correct L1 proteins.

The deficiencies of Browne et al are not compensated for by Danos et al. Danos is a U.S. Patent which claims various linear peptide sequences derived from HPV1 L1 and L2 genes. Also, this reference prophetically describes that these peptides may potentially be useful for protecting hosts against papillomavirus infection. The issued patent, however, contains no claims directed to such usages. Therefore, Danos et al this should not affect the patentability of the claimed invention which relates to conformationally correct L1 proteins. There is no disclosure whatsoever in Danos et al relating to conformationally correct proteins or their usage as HPV vaccines.

In this regard, it has been convincingly demonstrated that proper conformation of the L1 protein is essential for a proper (protective) immunogenic response against L1 and immunity to HPV infection. Therefore, the combination of Browne et al with Danos et al would not teach or suggest the claimed invention since neither reference teaches or suggests the production of conformationally correct HPV L1 proteins. Nor do either of these references recognize the criticality of proper conformation of the L1 sequence for obtaining a protective immune response. Quite clearly Danos et al completely fails to recognize the criticality of proper conformation since all of the exemplified peptides are linear peptides. The reference is further

deficient because the only disclosure relating to the use of the described peptides as potential vaccines is prophetic. Accordingly, based on the foregoing, withdrawal of the §103 rejection of Claims 13-14, 16-26 46-47 and 50-58 based on Browne et al taken in view of Danos et al is respectfully requested.

Claims 1-3, 10-12 and 15 stand rejected under 35 U.S.C. §102(b) as being anticipated by, or in the alternative, under 35 U.S.C. §103 as being obvious over Carter et al.

Essentially, the Examiner states that Carter et al discloses the expression of the L1 open reading frame of various human papillomavirus types i.e., HPV-1 and HPV-16 (as well as HPV 6). The Examiner further acknowledges that Carter et al does not characterize the recombinantly produced L1 proteins as having the properties of the cloned L1 proteins (include proper conformation). However, the Examiner concludes that it is reasonable to assume that such properties are "inherent" absent evidence to the contrary. Moreover, the Examiner further concludes that it would be reasonable to assume that the L1 proteins obtained using the methodology of Carter et al would inherently possess the same immunogenic properties as the claimed proteins. However, based on the following, this conclusion can not be sustained.

As discussed *supra*, and as substantiated by the §132 Declaration of Drs. Jenson and Schlegel, the cloning and expression methods of Carter et al would not give rise to conformational human papillomavirus L1 proteins. Essentially, for different reasons, each of the HPV-1, HPV-6(b) and HPV-16 L1 sequences

which were expressed by Carter et al would be reasonably expected not to result in conformationally correct HPV L1 proteins.

With respect to the HPV-16 L1 sequence expressed by Carter et al, the authors expressed the same defective mutant HPV-16 L1 DNA expressed by Browne et al (Id.) Minson (Id.) and Zhou (Id.). This is readily apparent based on their disclosed source of the HPV-16 L1 reading frame (Dürst et al, *Proc. Natl. Acad. of Sci., USA* 80:3812-3815 (1983) and Seedorf et al, *Virology* 145:181-185 (1985)). As discussed in Applicants March 8, 1995 Reply, Dürst et al (Id.) disclosed and made available the prototype L1 sequence which was expressed by Zhou et al. This sequence was derived from an HPV-16 genome which integrated into the chromosomes of cervical cell carcinoma. This prototype HPV-16 L1 DNA differs from the wild-type HPV 16 L1 DNA because it contains a point mutation at position 202 which converts an aspartic acid residue to a histidine residue in the resulting protein. Moreover, this single modification has substantial adverse effects on both conformation and proper viral particle assembly. In particular, as discussed in Roden et al (*Id.*), the HPV-16 L1 mutant assembles three orders of magnitude less efficiently than the wild-type HPV-16 DNA. Further, and more significantly, HPV-16 proteins obtained by expression of this mutant HPV-16 L1 sequence do not exhibit proper conformation. This is evidenced, e.g., by the last page of Roden et al wherein the authors note that antisera raised against particles obtained by expression of the HPV-16 prototype fail to prevent binding of wild-type HPV-16

virus-like particles to cell surfaces. Therefore, this mutation results in L1 proteins having altered conformation.

The fact that the HPV-16 prototype L1 sequence does not result in conformationally correct human papillomavirus L1 proteins is further substantiated by the Jenson §132 Declaration submitted with the March 8, 1995 Reply. This Declaration describes an immunofluorescence assay wherein the prototype HPV-16 L1 sequence and the wild-type L1 sequence were both expressed in Sf9 insect cells (using a baculovirus expression system) and tested for their reactivity with monoclonal antibodies. More specifically, the ability of the resultant L1 proteins to bind to six conformational antibodies, i.e., antibodies specific to such HPV-16 conformational epitopes and one antibody specific for a linear epitope was compared by immunofluorescence. These results demonstrated that all of the seven antibodies bound to the cells which expressed wild-type HPV-16 L1 proteins. By contrast, cells which expressed the prototype HPV-16 proteins failed to react with any of the six tested conformational antibodies.

Moreover, this was despite the fact that the prototype HPV-16 L1 was synthesized, transported into the nucleus and aggregated in a manner similar to the wild-type HPV-16 L1 proteins as shown by its reactivity with a linear non-conformationally-dependent HPV-16 L1 epitope. Thus, while the prototype HPV-16 L1 protein aggregates similar to the wild-type L1 protein, it fails to react with antibodies which recognize conformationally correct HPV-16 L1 epitopes. Thus, there is substan-

tial evidence of record (which has been found to be persuasive by the Examiner) which establishes that the prototype HPV-16 L1 DNA which was expressed by Carter et al does not give rise to conformationally correct HPV-16 L1 proteins.

Turning now to the HPV-6b sequence expressed by Carter et al, this sequence also would not be expected to give rise to conformationally correct L1 proteins. This is because Carter et al expressed a truncated HPV-6b sequence. This is apparent, for example, from page 515 of the reference wherein the authors state that the HPV-6b L1 sequence which was expressed encodes a 461 C-terminal amino acid sequence which comprises part of the entire 500 amino acid sequence. Therefore, the HPV-6b protein which was expressed by Carter et al lacks 39 of the N-terminal amino acids of the native HPV-6B L1 protein. Also, this truncated HPV-6B L1 sequence was expressed as a fusion with a 9 amino acid sequence (MGIRARYPG). Therefore, given both the fact that Carter et al expressed a truncated sequence lacking 39 N-terminal amino acids and further because they expressed it as a fusion protein, it is reasonable to assume that they did not obtain conformationally correct HPV-6b L1 proteins. In this regard, subsequent experiments conducted by the present inventors have demonstrated that the N-terminal portion of the L1 protein is essential for proper conformation of the L1 protein. Also, because it was expressed as a fusion protein, this would further likely adversely affect the conformation of the resultant truncated HPV-6b L1 protein. Therefore, for either or both of these reasons, the HPV-6b L1 proteins expressed by Carter et

al would reasonably be expected not to comprise proper conformational structure.

Finally, turning now to the HPV-1 L1 sequence expressed by Carter et al, Applicants respectfully advise that Carter et al cloned the HPV-1 L1 sequence by polymerase chain reaction (PCR) cloning methods. However, Carter et al took no precautions to ensure that the resultant cloned HPV-1 L1 sequence did not contain errors prior to expression. For example, the authors did not sequence their HPV-1 L1 DNA to identify any errors introduced by PCR. This is notwithstanding the fact that PCR typically gives rise to mutations.

PCR cloning techniques have an inherent infidelity (because of the enzyme used for amplification) and therefore typically give rise to sequence mutations every 300 to 500 base pairs. Consequently, given that the size of the HPV-1 L1 DNA which was cloned (about 1500 nucleotides), it would be reasonably expected that the cloned HPV-1 L1 DNA would contain about 3 to 5 errors, and possibly more.

The exact mutations probably introduced by PCR cloning are impossible to assess because Carter et al did not sequence their HPV-1 DNA. Moreover, it is further impossible to demonstrate by subsequent experiments the specific errors likely introduced because PCR is inherently unpredictable. However, given the high level of fastidiousness of the L1 protein, it would be reasonable to expect that at least one of the mutations which was likely introduced by PCR cloning would have adversely affected the conformation of the resultant protein. It is also

noted that the effect of such probable mutations introduced by PCR cloning is further impossible to assess from Carter et al because they did not conduct any experiments which would confirm proper conformation or lack thereof. The only way that proper conformation of L1 proteins can be confirmed is based on their reactivity with conformation-dependent antibodies. Conformation-dependent antibodies are antibodies which recognize epitopes expressed on conformationally correct L1 proteins. However, Carter et al conducted no immunological assays with conformation-dependent antibodies. Rather, the antibodies they used were directed to linear epitopes. However, even assuming *arguendo* that they had conducted the appropriate immunological assays, it is reasonable to assume that the expressed proteins would not have reacted with conformation-dependent antibodies because of the substantial flaws in their cloning and expression methods. Such a conclusion is supported by recent experiments conducted by the present inventors relating to expression of COPV-L1 carboxy terminal deletion mutants. In these experiments, the inventors used PCR cloning techniques to clone the COPV-L1 carboxy terminal deletion mutants. These results confirmed that PCR cloning methods typically resulted in significant mutation as determined by nucleotide sequencing.

Thus, based on the foregoing, it is believed to be clear that Carter et al does not teach or suggest the production of conformationally correct HPV L1 proteins notwithstanding the fact that they express three different HPV L1 sequences. Therefore, in total, Carter et al disclosed three different means of

expressing L1 proteins, two of which based on the information provided could not result in the formation of conformationally correct L1 proteins, and the third of which would reasonably not result in the formation of conformationally correct L1 proteins. Therefore, withdrawal of the §102(b) rejection and §103 rejection of Claims 1-3, 10-12 and 15 is respectfully requested. Essentially, given the above arguments, all of which are supported by evidence, it would be unreasonable to conclude that the L1 proteins described in Carter et al would inherently possess proper conformationall correct structure.

Claims 13, 14, 16-26, 46-47 and 50-58 stand rejected under 35 U.S.C. §103 as being unpatentable over Carter et al as applied to Claims 1-3, 10-12 and 15 above and further in view of Danos et al (U.S. Patent No. 4,551,270). Carter et al has been discussed *supra*. For the reasons set forth therein, the cloning methods in this reference reasonably would not be expected to result in HPV L1 proteins which reproduce the immunogenicity and conformation of L1 proteins expressed on the virion of intact native human papillomavirus virions.

With respect to the method claims, the Examiner has acknowledged that Carter et al does not teach or suggest a method of protecting humans against papillomavirus expression using the HPV L1 proteins produced in the reference. Rather, the reference merely suggests that HPV proteins expressed by yeast systems may be useful for examining humoral immunity to papillomaviruses and may serve as valuable tools for the study of cellular immunity and structure/function studies of the proteins

themselves. However, the reference contains no indication that L1 proteins could be used as effective vaccines for protecting against HPV infection.

The Examiner, however, has taken the position that this deficiency is cured by Danos et al. As discussed *supra*, Danos et al relates to linear peptide sequences derived from HPV-1 and prophetically describes that such peptides may be used in vaccine compositions. However, as previously established, neither linear HPV L1 proteins nor peptides are suitable for use as HPV vaccines. Rather, the expression of conformationally correct L1 epitopes is absolutely essential for an effective L1 protein, namely an L1 protein which may be used as a protective immunogen. Quite clearly, Danos et al does not express conformationally correct L1 proteins, nor does this reference recognize the criticality of conformation on an effective immunogenic response to HPV L1 proteins. Rather, the reference is entirely limited to the expression of linear peptides derived from HPV-1 surface proteins. Moreover, with respect to the linear peptides described therein, there is no evidence that such peptides could be used as an effective vaccine. To the contrary, the only disclosure in the patent relating to the use of HPV peptides as vaccines is prophetic.

Therefore, given the fact that neither Carter et al or Danos et al teaches the production of HPV conformationally correct L1 proteins, much less provide any indication that they would be useful as a vaccine, the method claims are clearly patentable over these references.

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Therefore, withdrawal of the §103 rejection of Claims 13, 14, 16-26, 46-47 and 50-58 based on Carter et al taken in view of Danos et al, is respectfully requested.

Based on the interview, it is believed that this Reply and the attached Declaration should be sufficient to remove all of the outstanding rejections. Therefore, a notice to that effect is respectfully solicited. However, if any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

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